LABORATORY BULLETIN

MONTANA STATE DEPARTMENT OF HEALTH AND ENVIRONMENTAL SCIENCES

HELENA, MONTANA

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MICROBIOLOGY IN THE STATE LABORATORY

THE MANUAL FOR STATE LABORATORIES IS "DIAGNOSTIC PROCEDURES FOR BACTERIAL, MYCOTIC AND PARASITIC INFECTIONS" FIFTH EDITION 1970 PUBLISHED BY BOOK SERVICE, AMERICAN PUBLIC HEALTH ASSOCIATION, 1015 18th St. N.W., Washington D.C. 20036. IT SHOULD BE AVAILABLE TO EVERYONE SENDING SPECIMENS TO THE STATE LABORATORY BECAUSE IT CONTAINS INSTRUCTIONS FOR THEIR COLLECTION AND SHIPMENT AS WELL AS INFORMATION USEFUL FOR INTERPRETATION OF RESULTS.

The functions of the state laboratory are to provide leadership and guidance to clinical laboratories through a laboratory improvement program which includes registration, certification, inspection and approval of laboratories and personnel; and to provide services in support of programs in the department. Reference microbiology accounts for 53 per cent ($101,772.00 in '71) of our services and is part of the Disease Control Program.

The purpose of this bulletin is to provide guidlines for submitting cultures to the state laboratory and also to list specimens which we will no longer accept routinely because they are clinical microbiology which should be done in the laboratory of your consulting pathologist or hospital.

SALMONELLA, ARIZONA, SHIGELLA

We are no longer accepting mixed cultures on solid agar or in liquid media for identification of enteric pathogens. If it is not possible to send pure cultures on agar stab/slants (which is the recommended procedure) then it is preferable to send the original clinical material--either a rectal swab in Stuart"s Transport Medium (or similar semi-solid medium) or a stool specimen in 30% buffered glycerol solution. Both of these transport media are available from the State Laboratory. These organisms grow well on any nutrient agar. Use an inoculation wire and pick a single suspected colony or pure culture. Stab the agar slant all the way to the bottom of the tube and then streak the surface of the slant. In this way the culture should still be alive within the stab even though it might die out on the slant. Screw cap tubes are most satisfactory but tubes which are sealed with corks which have been dipped in hot paraffin are also good. Do not dip the top of the tube in melted paraffin after the cork has been inserted in the tube. (We do not supply agar for transport.) A pure culture in Stuart's Transport Medium can also be submitted if no agar slants are available. Triple Sugar Iron Agar slants are not as satisfactory for transport since fermentation of the sugars in the medium may kill the bacteria. If it is necessary to use TSI please send a fresh culture. Do not send pure cultures on agar plates except in an emergency. Moisture tends to

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collect in plates during transit and carry bacteria out into the packing material. There is no sure way to keep an agar plate from leaking and this is hazardous to people receiving the mail.

ENTEROPATHOGENIC E. COLI

We do not process rectal swabs or stool specimens for enteropathogenic E. coli. However, isolated strains which have been screened with polyvalent OB serum (Difco is a source for this) may be sent for further study. The pure culture should be sent in the manner described above under Salmonella.

THROAT CULTURES FOR BETA HEMOLYTIC STREPTOCOCCI

This is part of the RHEUMATIC FEVER PREVENTION project of the Disease Control Division. Laboratory aspects have been discussed in Bulletins 25 and 30. One goal is to have throat cultures for Group A streptococci done on every case of pharyngitis in persons through age 22. If these are not done locally, swabs should be submitted in a packet of dry silica gel. These packets are available from the state laboratory and have been assembled especially for this project. A less satisfactory method is to submit the swab in Stuart's Transport Media. DO NOT send throat swabs for streptococci on Loeffler's Medium because it does not encourage the growth of streptococci but does encourage that of contaminants which easily overgrow any streptococci present. It is a good medium for C. diphtheriae.

For submission of isolated streptococci for grouping by the precipitin test, pick an isolated colony to an agar slant (Trypticase Soy Agar, Tryptose Blood Agar Base, or a similar highly nutrient agar). A dextrose-free agar should be used because dextrose promotes acid formation with the result that the organism may not survive the trip to the laboratory.

DO NOT SEND AGAR PLATES through the mail. Moisture collects in them during transit and carries bacteria out into the packing material. There is no sure way to keep an agar plate from leaking and this constitutes a hazard to people receiving the mail.

THROAT CULTURES FOR CORYNEBACTERIUM DIPHTHERIAE

For laboratories not doing their own isolation and identification, a throat swab should be sent in on Loeffler's Medium. This is the medium of choice for C. diphtheriae. Loeffler's can be obtained commercially or, in an emergency, from the State Laboratory. Or, if time doesn't permit, submit a swab in a small amount of broth (Brain Heart Infusion or comparable media) and send to the State Laboratory by the fastest method. It is not necessary to incubate the Loeffler's Medium before shipment since the organism grows quite well at room temperature. The State Laboratory should be informed by telephone that the specimen is coming so special media can be prepared and the specimen can be handled as an emergency if indicated.

GONORRHEA (See Bulletin No. 35)

At present the most satisfactory method for transport of specimens for Neisseria gonorrhoeae is the use of Martin-Lester "Transgrow" Medium which can be obtained either commercially or from the State Laboratory.

Martin-Lester "Transgrow" Medium is not intended for use within the laboratory for isolation of Neisseria gonorrhoeae. Thayer-Martin plates, obtainable commercially, are still the medium of choice for this purpose.

It is essential that the directions which accompany the "Transgrow" Medium be strictly followed. Please remember that the bottles have an atmosphere of carbon dioxide in them. Since this is heavier than air the bottles must be kept in an upright position when the caps are removed. Inoculation should be done quickly in the recommended "Z" pattern. If there is any amount of condensed water in the bottom of the bottle it is suggested that the swab first be dipped into this liquid so that it is removed. Then continue to streak the agar. If any liquid is left in the bottle it will distribute the organisms over the entire agar surface producing confluent growth and obviate the possibility of picking isolated colonies. The agar must be warmed to approximately 35-37° C. before streaking. The bottles should be incubated overnight before shipping to the State Laboratory so that the organisms will have grown enough to withstand the unfavorable environment of the mails. Should the CO_2 accidently be lost from the bottles, incubate after streaking in a candle jar, with the caps loose. Then tighten the caps securely immediately upon opening the candle jar. Neisseria gonorrhoeae seldom survives longer than three days so please ship specimens to arrive at the State Laboratory during that period. Mail is received and processed every day except Sunday. However, please try to ship specimens so they may arrive before the week-end.

ANAEROBES

Unless your laboratory is set up to grow anaerobic bacteria and to pick pure cultures to transport medium, it is best to send us the original clinical material--on a swab, if collected that way--in Stuart's (or similar semi-solid transport medium). Do not use any Stuart's Medium which is blue since this means oxygen is diffusing into the medium, a situation detrimental to anaerobes.

If you are able to pick pure isolates to transport medium then please use a medium designed for anaerobes such as thioglycollate medium (preferably without dextrose) or cooked meat medium in screw cap tubes. To prevent exposure of the medium to air it is essential to cover it with Vaspar. This is a mixture of 50% paraffin and 50% petrolatum (Vaseline). Melt and mix in a small tin can, pour into tubes, and sterilize in the autoclave. The procedure to be followed:

1. Pick the suspected colony to thioglycollate or cooked-meat medium. Both must be freshly made or heated in a boiling water bath for 10 minutes followed by immediate cooling in cold water and re-heating to 37° C. Do not use thioglycollate medium which is over two weeks old. Cooked meat will last longer.

2. Incubate at 37° for 18-24 hours. If dextrose (glucose) is present in the medium and the organism is a profuse gas former (such as Clostridium perfringens) then permit the gas to subside before proceeding to the next step.

3. Heat the Vaspar until barely melted and pour over the culture

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to a depth that will bring it up to the constriction at the top of the tube. This will keep the Vaspar firmly in place during transit. If Vaspar is too hot when poured over the medium it will tend to produce peroxides which are detrimental to anaerobes. The same thing is true of heating the medium before use--it must be no longer than ten minutes and must be done only once. Discard any unused heated tubes of media. Do not seal with paraffin alone; it is too hard and tends to shrink slightly on cooling so leakage will occur.

4. Ship immediately to the State Laboratory preferably not to be delayed by a week-end.

We are no longer accepting mixed cultures or agar plates for separation of anaerobes. In the first place, plates sent through the mail are exposed to oxygen in transit and most anaerobes will not survive the trip. Secondly, we cannot determine the relative numbers of organisms in the original specimen from cultures which have already been carried through several procedures by some other worker.

For those laboratories sending us the original clinical material in Stuart's it must be remembered that at this stage of the game there is no ideal way to transport specimens or anaerobic cultures. The anaerobes we do isolate under these conditions are the tough ones. Culturing for the most fastidious ones must be done at the bedside.

BACTERIAL FOOD POISONING

It is the responsibility of the sanitarian or other person conducting the investigation to procure the suspected vehicle as soon as possible. The suspected food should be cultured immediately in the nearest competent laboratory since bacterial populations rapidly change with time; and cold storage is detrimental to some of the organisms associated with food poisoning. However, if there is no avoiding a delay between collection and testing then shipping with dry ice is the lesser of two evils.

Most important to remember in the case of Staphylococcus aureus, Clostridium perfringens, and Salmonella food poisoning is that BOTH THE SUSPECTED FOOD AND FECES FROM PATIENTS SHOULD BE SENT. It is important to correlate the toxic strains of Staphylococcus and Clostridium from both food and feces in order to establish that the organism in the food is indeed the one that has affected the patient. In the case of Salmonella it is only necessary to establish that the bacterium is isolated from the food; but it is added evidence if the same organism is isolated from the patient.

Very often the suspected food is no longer available. In this case it is unnecessary to submit stools if Staphylococcus or Clostridium perfringens are suspected since Clostridia and Staphylococci (to a lesser degree) are normally found in the stool. Without correlation of toxin types between food and stool there is no point in culturing stools for these two organisms.

Salmonella, on the other hand, is not normal in the stool. Thus, even if the suspected food is not available the presence in the stool of Salmonella is significant.

CHOLERA

According to a recent release from the Center for Disease Control in Atlanta, Georgia, cholera need no longer be considered a dread disease. Severe cases respond well to intravenous fluid and electrolyte replacement therapy. Milder cases can be treated with either intravenous or oral fluid therapy and tetracycline. However, studies have shown that for each person who becomes sick enough for his case to be diagnosed and reported, there may be as many as 25 to 100 with mild or asymptomatic infections. It is this latter group that may unwittingly transport the disease to new and distant foci. Air travel has now added a new dimension and with the spread of cholera, cases may occur in the United States.

Physicians should consider the possibility of cholera in any patient who has diarrhea within five days after returning from an infected area.

Those laboratories doing their own bacteriology will find thiosulphate citrate bile salts sucrose agar (TCBS) or tellurite taurocholate gelatin agar(TTGA) useful. And for enrichment, alkaline peptone water.

For transport of rectal swabs to the State Laboratory, Stuart's Transport Medium can be used or a portion of stool can be sent in alkaline peptone water if it is available. In an emergency, a piece of blotting paper dipped into the stool and inserted into a leak-proof plastic bag or screw-capped jar will do.

(ABOVE TO BE CONTINUED)

CURRENT NOTES

1. Hospital Microbiology

Some hospitals which should have a complete bacteriological service do not because of lack of support by physicians in the area. The Montana Hospital Licensing Law sets up the following classifications:

1. General Hospital - 100 beds or more
2. Intermediate General Hospital - 40-99 beds
3. Small General Hospital - 10-39 beds

With proper outpatient use of their services, hospitals of 100 beds should be able to afford a full-time clinical bacteriologist. The state laboratory will do its best to avoid competing with hospital laboratories. However, we cannot deny physicians and their patients access to quality microbiology and will do everything within reason to assure that it is available locally or through their consulting pathologist.

In addition to Clinical Microbiology we must consider bacteriology from the standpoint of INFECTION CONTROL. The following was written by a former laboratory surveyor for Montana:

"Because of the importance of infections in hospitals, both from a medical and economic standpoint, the laboratory should take an active part in their control. The laboratory must supply the data on which an Infection Control Program is based. No hospital is exempt from the problem of nosocomial infections, therefore every

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laboratory must have a minimum capability to perform the bacteriological examinations required for control of infections. It should be able to:

a. Isolate and perform the initial identification of organisms from hospital patients with infections

b. When indicated, take cultures of the hospital environment, and quantitate and identify organisms isolated. (Such cultures should be performed when the Infection Committee establishes a meaningful surveillance program and when required by regulation or law.)

c. Evaluate results and discuss them with the Infection Committee and other hospital personnel as necessary."

2. Revision of COSTS as listed in Bulletin #36

It is very important when entrusted with the expenditure of public funds to give a true picture of the cost of the services provided. Therefore, we continually examine the schedule presented in Bulletin 36. Recently, Iowa and Maryland questioned our cost of $0.60 for Rubella hemagglutination and upon recalculation we found the following (based on 10,000 tests annually):

(pro-rated per test)	reagents	0.18
	chick rbc for absorption and testing	0.10
	labor (includes glassware washing & specimen preparation)	0.65
	postage, handling, and reporting (a charge against all specimens)	0.35
	TOTAL	$1.28